

In the Claims

1 (currently amended). A method for purifying recombinant human follicle stimulating hormone (FSH) ~~FSH~~ or an FSH variant comprising the steps of subjecting FSH to:

- (1) ion exchange chromatography;
- (2) immobilised metal ion chromatography; and
- (3) hydrophobic interaction chromatography (HIC).

2 (original). The method of claim 1, wherein the ion exchange chromatography is carried out with a strong anion exchange resin.

3 (original). The method of claim 2, wherein the anion exchange resin is Q Sepharose FF, or a resin having similar properties.

4 (currently amended). ~~The method of any one of claims 1 to 3~~ method of claim 1, wherein the ion exchange chromatography is carried out using borate buffer as eluent.

5 (original). The method of claim 4, wherein the borate buffer is at a pH of at or about 8.5.

6 (currently amended). ~~The method of any one of claims 1 to 5~~ method of claim 1, wherein the immobilised metal ion chromatography is carried out with a resin having tridentate chelating groups.

7 (original). The method of claim 6, wherein the chelating groups are iminodiacetic acid.

8 (currently amended). ~~The method of any one of claims 1 to 7~~ method of claim 1, wherein the immobilised metal ion chromatography is carried out with chelating Sepharose FF, or a resin having similar properties.

9 (currently amended). ~~The method of any one of claims 1 to 8~~ method of claim 1, wherein the immobilised metal ion chromatography is carried out with a metal ion selected from Cu^{2+} , Zn^{2+} , Ni^{2+} , Ca^{2+} , Mg^{2+} and Co^{2+} .

10 (currently amended). ~~The method of any one of claims 1 to 8~~ method of claim 1, wherein the immobilised metal ion chromatography is carried out with Cu^{2+} .

11 (currently amended). ~~The method of any one of claims 1 to 10~~ method of claim 1, wherein the immobilised metal ion chromatography is carried out using ammonium acetate as eluent.

12 (original). The method of claim 11, wherein the ammonium acetate buffer has a pH of at or about 9.

13 (currently amended). ~~The method of any one of claims 1 to 12~~ method of claim 1, wherein the hydrophobic interaction chromatography (HIC) is carried out using Phenyl Sepharose FF HS, or a resin having similar characteristics.

14 (currently amended). ~~The method of any one of claims 1 to 13~~ method of claim 1, wherein the hydrophobic interaction chromatography is carried out using ammonium acetate (50 mM)/ammonium sulphate (0.25 M) as eluent.

15 (currently amended). ~~The method of any one of claims 1 to 14~~ method of claim 1, comprising a second step of ion exchange chromatography (2a), carried out after the step of immobilised metal ion chromatography, and before the step of hydrophobic interaction chromatography (HIC).

16 (original). The method of claim 15, wherein the second step of ion exchange chromatography is carried out using a weak anion exchange resin.

17 (original). The method of claim 16, wherein the weak anion exchange resin is DEAE Sepharose FF resin, or a resin having similar properties.

18 (currently amended). ~~The method of any one of claims 1 to 17~~ method of claim 1, further comprising a step of reverse phase chromatography (4), carried out after the step of hydrophobic interaction chromatography (HIC).

19 (original). The method of claim 18, wherein the reverse phase chromatography is carried out using Source 30 RPC as resin, or a resin having similar characteristics.

20 (original). The method of claim 19, wherein the reverse phase chromatography is carried out using ammonium acetate (50 mM, pH at or about 7.6) with 20% (v/v) 2-propanol.

21 (currently amended). ~~The method of claim 18, 19 or 20~~, comprising a step of ultrafiltration (5), carried out after the step of reverse phase chromatography.

22 (currently amended). A method for purifying human recombinant FSH comprising the steps of subjecting FSH to:

- ~~(0)~~ (i) ultrafiltration;
- ~~(1)~~ (ii) anion exchange chromatography on Q Sepharose FF with at or about 50 mM borate, at or about 0.13 M NaCl, pH at or about 8.5 as eluent;
- ~~(2)~~ (iii) subjecting the eluate of step ~~(1)~~ (ii) to a step of immobilised metal ion affinity chromatography on chelating Sepharose ff, with Cu^{++} as metal ion, and at or about 0.75 M ammonium acetate pH at or about 9 as eluent;
- ~~(2a)~~ (iv) subjecting the eluate of step ~~(2)~~ (iii) to a step of anion exchange chromatography on DEAE Sepharose FF, with at or about 0.11 M Ammonium acetate, pH at or about 8.5 as eluent;

- (3) (v) subjecting the eluate of step ~~(2a)~~ (iv) to a step of hydrophobic interaction chromatography on Phenyl Sepharose FF HS with at or about 50 mM ammonium acetate, at or about 0.25 M ammonium sulphate, pH at or about 8.25 as eluent;
- (4) (vi) subjecting the eluate of step ~~(3)~~ (v) to a step of reverse phase chromatography on Source 30 RPC, with at or about 50 mM ammonium acetate, pH at or about 7.6, with at or about 20% of 2-propanol (v/v);
- ~~(5)~~ (vii) subjecting the eluate of step ~~(4)~~ (vi) to a step of ultrafiltration; and
- ~~(6)~~ (viii) subjecting the retentate of step ~~(5)~~ (vii) to a step of nanofiltration.

23-24 (canceled).

25 (new). A composition of matter comprising:

- (a) a purified recombinant human FSH or FSH variant produced by the process of claim 1; or
- (b) a composition comprising a purified recombinant human FSH or FSH variant produced by the process of claim 1 and a liquid.

26 (new). The composition of matter of claim 25, wherein said liquid is a buffer, stabilizer or an excipient.